Aquaculture 473 (2017) 337–344

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Evaluation of black soldier fly (*Hermetia illucens*) larvae meal as partial or total replacement of marine fish meal in practical diets for Pacific white shrimp (*Litopenaeus vannamei*)

Vaun C. Cummins Jr [a], Steven D. Rawles [b], Kenneth R. Thompson [a], Alejandro Velasquez [a,1], Yuka Kobayashi [a,2], Janelle Hager [a], Carl D. Webster [b,*]

[a] Aquaculture Research Center, Kentucky State University, Frankfort, KY, 40601, USA
[b] United States Department of Agriculture (USDA), Agricultural Research Service (ARS), Harry K. Dupree Stuttgart National Aquaculture Research Center (HKDSNARC), Stuttgart, AR, USA

ARTICLE INFO

Article history:
Received 26 October 2016
Received in revised form 17 January 2017
Accepted 16 February 2017
Available online 20 February 2017

ABSTRACT

Black soldier fly larvae (BSFL) meal, produced from the larvae of *Hermetia illucens,* has shown promise as a fish meal (FM) replacement in diets for rainbow trout, catfish and tilapia, but has not been examined as an alternative protein source in shrimp diets. Six isonitrogenous (35% crude protein, as fed) and isoenergetic (16.7 kJ available energy g^{-1} of diet) diets containing graded levels of BSFL as replacements for protein from menhaden FM were fed to juvenile (1.24 g ± 0.01; mean ± SE) Pacific white shrimp, *Litopenaeus vannamei.* Diet 1 (the control) was formulated similar to a commercial shrimp diet containing 25% menhaden FM and 23% soybean meal. Diets 2–6 were formulated as a dose-response series that progressively replaced protein from menhaden FM with BSFL meal at inclusion rates of 7%, 14%, 21%, 28%, and 36% of diet; this equated to progressively replacing 16.5% of dietary protein provided by menhaden FM. Diets were fed to juvenile shrimp stocked into eighteen 110-L saltwater aquaria (30 ppt) (three replicates per dietary treatment) at a density of 15 shrimp per aquarium (50/m^2) for 63 days. Nonlinear and spline regression analysis of responses indicated that the maximum level of BSFL meal inclusion varied significantly with the response being modeled. Generally, without modification of the ingredient or replacement diet nutrient profiles, 95% to 100% of most growth responses, i.e., shrimp final weight, weight gain, specific growth rate, and food conversion, could be obtained if replacement of FM by BSFL meal was limited to <25% of the diet, depending on performance measure. Similarly, 95% or greater of maximum whole-body protein and lipid content could be achieved when BSFL inclusion was restricted to <29% and 15%, respectively. Comparison of amino acid profiles in the test diets with recent requirement estimates for limiting amino acids in BSFL meal also suggest future strategies for increasing dietary substitution of FM with BSFL.

Published by Elsevier B.V.

1. Introduction

Global aquaculture production of shrimp has grown dramatically within the last two decades. Pacific white shrimp (*Litopenaeus vannamei*) generated US$17 billion from a production volume between 2.2 and 2.7 mmt in 2014, accounting for 15% of the total value of internationally-traded fishery products, and is currently the most valuable single aquaculture commodity (FAO, 2012; GlobeFish, 2015). Over 90% of farmed shrimp rely on high protein diets containing high percentages of marine fish meal (FM). Fish meal is used as the primary protein source in shrimp diets because of its favorable nutrient profile, indispensible amino acid and fatty acid composition, palatability, and relatively high digestibility (Cruz-Suarez et al., 2007; Lemos et al., 2009; NRC, 2011). However, a dichotomy exists between the rapid growth of the shrimp industry and static production of FM. Fish meal is a finite resource with limited availability and high demand, and therefore is the most expensive macro-ingredient in shrimp diets. In 2006, 27% of the FM in the aquaculture sector was utilized in shrimp diets (Tacon and Metian, 2008; Hardy, 2010), and while advances have been made in FM substitution and reduction in the last decade, overall expansion of shrimp production has subsequently led to an increase in the quantity of FM utilized (Naylor et al., 2009; Hardy, 2010).

Typically, the inclusion of FM in shrimp diets is 20–50% of the total diet formulation, which results in higher diet and production costs. Diet costs can account for between 50 and 80% of a producer's operational costs, and thus directly influences producer profitability. Future growth and profitability within the shrimp aquaculture sector is

* Corresponding author at: USDA/ARS - HKDSNARC USA, P.O. Box 1050, 2955 Highway 130 East, Stuttgart, AR 72160-1050, USA.
E-mail address: carl.webster@ars.usda.gov (C.D. Webster).
[1] Present Address: Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, Texas, 77843, USA.
[2] Present Address: 2001 Marine Dr., Room 253, Astoria, OR 97103, USA.

http://dx.doi.org/10.1016/j.aquaculture.2017.02.022
0044-8486/Published by Elsevier B.V.

dependent upon continued improvements in diet efficiency and formulation; specifically a reduction in the inclusion of expensive marine protein sources in shrimp diets. In addition, retailers and consumers have begun to consider both the environmental sustainability and health benefits of the foods they purchase, and there is growing concern regarding the sustainability of aquaculture practices that utilize high percentages of marine inputs, and/or deteriorate the natural environment.

In light of these economic, environmental and social concerns, a wide variety of renewable plant-based proteins have been evaluated in shrimp diets in an effort to reduce pressure and reliance on marine resources while increasing producer profitability. Several candidates for partially or totally replacing FM in diets for white shrimp have already been evaluated including lupin meal (Molina-Poveda et al., 2013); defatted microalgae meal (Ju et al., 2012); rendered animal-protein ingredient mixture (Ye et al., 2011); peanut meal (Liu et al., 2012); microbial floc meal (Bauer et al., 2012); plant-protein ingredient mixture (Suarez et al., 2009); and soybean-based diets (Sookying et al., 2013). However, there has been limited success in totally replacing FM in shrimp diets, depending on the nutrient quality and composition of the substitution ingredients, diet formulation, and culture system utilized (clear-water versus green-water) and often reduced growth and feed efficiency has resulted when FM was totally replaced by a single plant-protein ingredient (Lim and Dominy, 1990; Fox et al., 2004; Molina-Poveda and Morales, 2004; Alvarez et al., 2007; Suarez et al., 2009);

Among plant-protein alternatives evaluated thus far, soybean meal (SBM) is the most widely-used in aquaculture diets due to its wide availability, comparatively low market price, nutritional consistency, balanced amino acid profile, and high digestibility (Akiyama et al., 1989; Gatlin et al., 2007; Trosvik et al., 2012; Cummins et al., 2013). However, SBM also has negative attributes that may limit the level of diet inclusion or use as the sole protein ingredient in commercial shrimp diets. Lim and Dominy (1990), for example, reported reduced growth of shrimp fed levels of SBM >28% and attributed this to reduced feed intake and poor diet palatability. When compared to FM, SBM has lower essential amino acid (EAA) concentrations, particularly methionine, lysine, and threonine as well as a lack of essential n-3 fatty acids EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acids) (Fox et al., 2004; Gatlin et al., 2007; NRC, 2011). Moreover, soybean meal is known to contain anti-nutritional factors such as trypsin inhibitors, lectins, phytic acid, saponins, antivitamins, and high levels of non-starch polysaccharides and oligosaccharides that affect nutrient digestibility and/or availability to shrimp (Francis et al., 2001; Gatlin et al., 2007).

A potential candidate ingredient for use in combination with SBM is the black soldier fly (*Hermetia illucens*) which has been evaluated as a possible organism to use in bioconversion of manure to reduce waste residue. They can reduce nitrogen waste by 75% and reduce mass by 50% in poultry and hog operations (Newton et al., 2005). Further, the prepupae are highly nutritious comprised of 40% protein and 35% lipid (Bondari and Sheppard, 1981, 1987). Use of invertebrate protein sources as alternatives to fish meal in freshwater crustacean diets has been reported (Langer et al., 2011; Riddick, 2013). Thus, use of black soldier fly larvae (BSFL) meal for marine shrimp diets as FM replacement is warranted.

2. Materials and methods

2.1. Diet composition, preparation, and pellet stability

BSFL meal utilized in this experiment was obtained from a commercial producer (Enviroflight, Yellow Springs, Ohio USA) and contained 35% lipid. BSFL meal was extracted four times with a 2:1 (v/v) ethyl alcohol (95%) to BSFL meal for each extraction, followed by one extraction of 3:1 (v/v), and a final extraction with a 4:1 (v/v) ratio (Table 1). During each extraction, BSFL meal was completely mixed with ethyl alcohol and allowed to settle for 10 min, followed by decanting of ethyl alcohol from the BSFL meal. After final extraction, solvent-extracted BSFL was air-dried under a fume hood for 24 h and then stored in a freezer (−20 °C) until needed.

Table 1
Proximate and selected amino acid composition (g kg^{-1} diet, as-fed basis) of menhaden fish meal (FM), and black soldier fly larvae (BSFL) meal.

	Ingredient		
	FM	BSFL	% difference[a]
Moisture	71.0	100.6	40.7
Protein	660.2	520.3	−21.2
Lipid	108.6	151.0	39.0
Ash	218.2	72.9	−66.6
Amino acids[b]			
Arginine	34.4	22.9	−33.4
Cystine	4.9	3.7	−24.5
Histidine	14.2	15.0	5.6
Isoleucine	26.4	18.7	−29.2
Leucine	32.5	32.3	−0.6
Lysine	48.0	27.1	−43.5
Methionine	16.2	6.6	−59.3
Phenylalanine	25.9	16.3	−37.1
Threonine	29.1	17.0	−41.6
Tryptophan	8.4	5.5	−34.5
Tyrosine	18.9	22.5	19.0
Valine	31.0	25.6	−17.4

[a] % difference calculated as $(AA_{BSFL} - AA_{FM}) / AA_{FM} * 100$, where AA_i is the amino acid concentration in ingredient *i*.
[b] Limiting order of essential amino acids in BSFL when compared to FM is Met, Lys, Thr, Phe, Trp/Arg.

Six isonitrogenous (35% protein, as fed) and isoenergetic (16.7 kJ available energy g^{-1} of diet) diets were formulated to meet the known nutrient and energy requirements of penaeid shrimp (NRC, 2011), with all experimental diets containing 23% SBM. Since a digestible energy value for BSFL meal has not been determined for white shrimp, available energy was calculated using physiological fuel values of 16.74, 16.74, and 37.66 kJ g^{-1} for carbohydrate, protein, and lipid, respectively (Webster et al., 1995). Proximate (Table 2) and amino acid (Table 3) compositions of the diets were measured by a commercial laboratory (Eurofins Scientific, Inc., Des Moines, IA, USA). Diet 1 (the control) was formulated to be similar to a commercially-available diet containing 25% menhaden fish meal (FM) and 23% soybean meal (SBM). Diets 2–5 were formulated to partially replace FM with BSFL at inclusion rates of 7%, 14%, 21%, and 28%, respectively, whereas, Diet 6 was formulated to completely replace FM with inclusion of 36% BSFL. Protein replacement of FM was equal to 0% (Diet 1), 20% (Diet 2), 40% (Diet 3), 60% (Diet 4), 80% (Diet 5), and 100% (Diet 6).

Dry ingredients were weighed (Mettler AT261 Delta Range, Mettler Instruments, Zurich, Switzerland) and mixed together for 1 h in a Hobart mixer (Hobart A-200, Troy, OH, USA). Warm tap water was added to the mash to obtain a 35% moisture level. Diets were double-extruded through a 2-mm die to form strands and then air-dried. After drying, diets were ground into pellets of appropriate size using a S.500 disk mill (Glen Mills Inc., Clifton, NJ, USA). Diets were sieved (2-mm opening mesh and 0.5-mm mesh) using a USA standard testing sieve (Fisher Scientific, Pittsburg, PA, USA). After sieving, a combination of soybean oil and menhaden fish oil that had previously been mixed together was added to the diets until all pellets were uniformly coated. Diets were stored in labeled plastic containers at −20 °C until fed.

The dried test diets were evaluated for pellet stability in water. Ten grams of pelleted diet of equal length were uniformly distributed on a 2-mm mesh screen sieve (Fisher Scientific, Pittsburg, PA, USA). Samples were then lowered into static saltwater approximately 10 cm deep for 30 min, dried in an oven (67 °C) for 24 h, and then cooled in a desiccator for 12 h prior to weighing. The residue left on the screen was recorded as dry solids not leached in water. The percentage of dry solids on the screen after 30 min in water to total solids in pellets was used as a comparative measure of pellet stability in water (Webster et al., 1994).

Table 2
Composition (g kg^{-1}) of six practical diets fed to juvenile white shrimp for 63 days containing graded levels of black soldier fly larvae (BSFL) meal as a replacement for menhaden fish meal (FM).

Ingredients	Diet 1 0BSFL	Diet 2 7BSFL	Diet 3 14BSFL	Diet 4 21BSFL	Diet 5 26BSFL	Diet 6 36BSFL
Menhaden fish meal	250.0	200.0	150.1	100.0	50.0	0.0
BSFL meal	0.0	70.7	141.3	212.0	282.6	363.3
Soybean meal (50%)	230.0	230.0	230.0	230.0	230.0	230.0
Wheat	380.7	365.0	349.4	328.7	303.1	277.4
Wheat gluten	50.0	50.0	50.0	50.0	50.0	50.0
Menhaden fish oil	10.0	15.0	20.0	25.0	30.0	35.0
Soybean oil	35.0	25.0	15.0	10.0	10.0	00.0
Dicalcium phosphate	15.0	15.0	15.0	15.0	15.0	15.0
Potassium phosphate	8.0	8.0	8.0	8.0	8.0	8.0
Magnesium sulfate	6.0	6.0	6.0	6.0	6.0	6.0
Lecithin	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin mix[a]	4.0	4.0	4.0	4.0	4.0	4.0
Cholesterol	3.0	3.0	3.0	3.0	3.0	3.0
Choline chloride	1.5	1.5	1.5	1.5	1.5	1.5
Mineral mix[b]	1.0	1.0	1.0	1.0	1.0	1.0
Stay C (35%)	0.7	0.7	0.7	0.7	0.7	0.7
Analyzed composition (dry-matter basis)[c]						
Crude protein, g kg^{-1}	426.6	418.2	415.2	407.8	403.0	401.5
Crude fat, g kg^{-1}	102.4	103.9	105.7	109.1	123.3	124.9
Ash, g kg^{-1}	93.2	93.8	85.5	79.5	74.9	72.0
Moisture, g kg^{-1}	84.4	87.3	84.1	82.2	81.7	87.5

[a] Vitamin mix supplied the following (mg or IU kg^{-1} of diet): biotin, 0.64 mg; B_{12}, 0.06 mg; E (as alpha-tocopherol acetate), 363 IU; folacin, 9.5 mg; myo-inositol, 198 mg; K (as menadion sodium bisulfate complex), 3.7 mg; niacin, 280 mg; D-pantothenic acid, 117 mg; B_6, 31.6 mg; riboflavin, 57.4 mg; thiamin, 35.8 mg; D_1, 440 IU; A (as vitamin A palmitate), 6607 IU.
[b] Mineral mix supplied the following (g kg^{-1} of diet): zinc, 0.07 g; manganese, 0.02 g; copper, 0.002 g; iodine, 0.010 g.
[c] Values are means of two replicates per diet.

Table 3
Amino acid composition (g kg^{-1} of diet, as-fed basis), squared differences (S_d) in individual amino acids concentrations, and sum of the squared differences (SS_d) in amino acid concentrations between the control diet (Diet 1; 0BSFL) and diets 2–6 containing graded levels of black soldier fly larvae (BSFL) meal as a replacement for menhaden fish meal (FM) in juvenile white shrimp diets. Values are means of two replicate determinations on each diet.

Amino acid		Diet 1 0BSFL	Diet 2 7BSFL	Diet 3 14BSFL	Diet 4 21BSFL	Diet 5 26BSFL	Diet 6 36BSFL
Alanine		19.6	19.5	19.3	19.0	19.0	19.1
	S_d	*0.0*	*0.01*	*0.09*	*0.36*	*0.36*	*0.25*
Arginine (19.6–23.2)		24.3	23.5	23.0	22.3	21.5	21.1
	S_d	*0.0*	*0.64*	*1.69*	*4.00*	*7.84*	*10.24*
Aspartic acid		36.1	35.4	34.9	34.1	33.5	33.5
	S_d	*0.0*	*0.49*	*1.44*	*4.00*	*6.76*	*6.76*
Cystine		5.2	4.6	4.7	4.7	4.6	4.5
	S_d	*0.0*	*0.36*	*0.25*	*0.25*	*0.36*	*0.49*
Glutamic acid		85.1	83.5	82.3	80.4	78.0	76.9
	S_d	*0.0*	*2.56*	*7.84*	*22.09*	*50.41*	*67.24*
Glycine		20.9	20.3	19.6	18.8	18.1	17.6
	S_d	*0.0*	*0.36*	*1.69*	*4.41*	*7.84*	*10.89*
Histidine		10.4	10.4	10.5	10.6	10.7	10.9
	S_d	*0.0*	*0.0*	*0.01*	*0.04*	*0.09*	*0.25*
Isoleucine		17.4	17.2	16.8	16.6	16.1	16.1
	S_d	*0.0*	*0.04*	*0.36*	*0.64*	*1.69*	*1.69*
Leucine		31.1	30.5	29.9	29.3	28.5	28.5
	S_d	*0.0*	*0.36*	*1.44*	*3.24*	*6.76*	*6.76*
Lysine (16.4–20.5)		27.4	27.9	25.7	24.9	24.4	24.0
	S_d	*0.0*	*0.25*	*2.89*	*6.25*	*9.00*	*11.56*
Methionine (6.6–9.1)		7.9	7.1	6.8	6.2	5.8	5.3
	S_d	*0.0*	*0.64*	*1.21*	*2.89*	*4.41*	*6.76*
Phenylalanine		19.4	18.8	18.2	17.7	17.0	16.6
	S_d	*0.0*	*0.36*	*1.44*	*2.89*	*5.76*	*7.84*
Proline		25.8	26.5	26.5	26.7	26.9	27.1
	S_d	*0.0*	*0.49*	*0.49*	*0.81*	*1.21*	*1.69*
Serine		19.2	18.9	18.9	18.6	18.6	18.5
	S_d	*0.0*	*0.09*	*0.09*	*0.36*	*0.36*	*0.49*
Threonine (11.8–15.1)		15.5	15.1	14.8	14.2	13.9	13.8
	S_d	*0.0*	*0.16*	*0.49*	*1.69*	*2.56*	*2.89*
Tryptophan		4.5	4.4	4.4	4.4	4.4	4.4
	S_d	*0.0*	*0.01*	*0.01*	*0.01*	*0.01*	*0.01*
Tyrosine		12.4	12.6	13.2	13.8	14.0	14.6
	S_d	*0.0*	*0.04*	*0.64*	*1.96*	*2.56*	*4.84*
Valine		19.4	19.5	19.3	19.3	19.1	19.3
	S_d	*0.0*	*0.01*	*0.01*	*0.01*	*0.09*	*0.01*
	SS_d	*0.0*	*6.87*	*22.08*	*55.90*	*108.07*	*140.66*

2.2. System components and water quality

The feeding trial was conducted at the Aquaculture Research Center, Kentucky State University, Frankfort, Kentucky USA in an indoor, recirculating, saltwater system comprised of 18 aquaria (110-L each). Crystal Marine® salt mix (Marine Enterprises International, Baltimore, MD, USA) was added to dechlorinated city (tap) water to obtain salinity levels equivalent to seawater (30 ppt). Saltwater was recirculated through a 2000-L mechanical and biological filtration system containing vertical polyester screens and polyethylene bio-balls (Red Ewald, Karnes City, TX, USA) for solids removal and fixed-film nitrification. Water then passed through a propeller-washed bead filter (Aquaculture Systems Technologies, New Orleans, LA, USA), which provided substrate for nitrifying (*Nitrosomonas* and *Nitrobacter*) bacteria and aided in the removal of additional nitrogenous wastes before the water circulated back to the aquaria. Water was supplied to each aquarium at a rate of 4.0 L/min. An immersion heater located in the biological and mechanical filtration system was used to maintain optimal water temperature. Continuous aeration was provided by a Rotron blower (Ametek, Kent, OH, USA), which supplied atmospheric oxygen to a single 4-inch airstone in each aquarium and additional airstones in the biological and mechanical filtration components. To ameliorate water-loss from evaporation and routine maintenance (siphoning), approximately 5% of the total water volume was replaced daily using a combination of dechlorinated city (tap) water and high salinity water (40 ppt) held in separate 380-L storage tanks. The photoperiod was provided by overhead fluorescent ceiling lights set on a 14 h:10 h light:dark cycle. All aquaria were siphoned daily to remove uneaten diet and feces. Mortalities and molting were recorded daily, and molts removed upon notice. All tanks were covered with polyethylene mesh to prevent access to adjacent aquaria and/or losses from jumping.

Dissolved oxygen, salinity, pH, and water temperature were measured daily using a Hydrolab Quanta Water Quality Monitoring System: Model QD 02152 (HACH, Inc., Loveland, CO, USA). Total ammonia nitrogen (TAN), nitrite levels, and pH were recorded three times per week using a HACH DR 2800 spectrophotometer (HACH, Inc.). Water quality parameters measured in the experimental system for the duration of the trial were optimal for growth and survival of white shrimp and averaged: temperature (29.5 °C), salinity (30.7 ppt), dissolved oxygen (5.16 mg L^{-1}), pH (7.9), total ammonia nitrogen (0.13 mg L^{-1}), and nitrite (0.10 mg L^{-1}) for the duration of the study.

2.3. Shrimp stocking and feeding

Juvenile *Litopenaeus vannamei* were obtained from Shrimp Improvement Systems (Islamorada, FL, USA) and acclimated to the salinity and temperature of the experimental system by gradually mixing the system water with the holding water so that environmental changes did not exceed 1 °C and 1 ppt salinity every 30 min. To ensure that all shrimp were nutritionally equivalent and that a nutritional baseline was established, a maintenance/conditioning diet was fed two times per day for 7 days prior to the beginning of the study. Once acclimated, juvenile shrimp (1.24 ± 0.01; mean ± SE) were randomly selected and batch-weighed to determine an initial average weight. Shrimp were stocked randomly into 18 110-L aquaria at a density of 15 shrimp/aquarium (50 m^{-2}). Mortalities were replaced during the first week of the feeding trial.

Shrimp were fed one of the six test diets with three replicates per dietary treatment, and were hand-fed four times per day at 0730, 1030, 1330, and 1630 for 63 days. Aquaria biomass and daily feed rations were adjusted for mortalities, and the amount of diet fed per day was recorded for each aquaria.

2.4. Data analysis

At the conclusion of the feeding trial, shrimp were harvested, chill-killed in an ice-water bath to drastically reduce body temperature, then weighed, placed into plastic storage bags and frozen for subsequent whole-body amino acid and proximate analysis. Response parameters were as follows:

Final individual weight (g/shrimp) = $W_{f;}$

Percent weight gain (%) = $[(W_f - W_i) \times 100] / W_i$ where W_i = initial individual weight (g/shrimp);

Specific growth rate (SGR) = $(\ln W_f - \ln W_i \times 100) / t$ where t is time in days;

Feed intake (g/shrimp);

Feed conversion ratio (FCR) = dry feed intake (g)/final weight gain (g);

Percent survival (S, %) = $(N_f/N_i) \times 100$, where N_i and N_f are the initial and final number of shrimp, respectively.

Linear, quadratic and spline regression models were fit to the responses of whiteleg shrimp to BSFL meal dietary inclusion level using the software procedures PROC REG or PROC NLIN in SAS 9.3 (SAS Institute, Inc., Cary, NC, USA). The most parsimonious model for each response was chosen that yielded practical dietary BSFL concentrations (i.e.,≥0) with the minimum mean square error (MSE), maximum adjusted R^2 according to Kvalseth (1985), and smallest *P*-value. Regressions were considered significant when $P \leq 0.05$ and $R^2 \geq 0.25$. To examine the potential influence of diet amino acid profile on fish responses, the distance between the control diet (Diet 1; 0BSFL) amino acid profile and that of the other five test diets containing graded levels of BSFL meal was calculated, where distance was defined as the sum of the squared deviations (SS_d) in individual amino acid concentrations between the control diet (Diet 1) and each of the other test diets (Diets 2–6). For example, in Table 3, Arg concentration was 24.3 g kg^{-1} in the control diet and 21.1 g kg^{-1} in Diet 6. The squared deviation (S_d) of Arg in Diet 6 from the control diet is therefore $(24.3–21.1)^2 = 10.24$. Similarly, the squared deviation of Lys in Diet 6 from the control diet D1 is $(27.4–24.0)^2 = 11.56$, etc. Hence, the sum of squared deviations (SS_d) in amino acid concentrations of Diet 6 from the control diet is $0.25 + 10.24 + \ldots + 11.56 + \ldots + 0.01 = 140.66$ (Table 3).

3. Results

Composition of the two protein sources of interest, FM and BSFL meal, were markedly different (Table 1). Moisture and lipid content of BSFL were nearly 41% and 39% higher, respectively, than FM, whereas protein and ash content of BSFL were 21% and 67% lower, respectively, than FM. With the exception of His and Tyr, concentrations of all other amino acids measured in BSFL meal were lower than those of FM. The limiting order of essential amino acids in BSFL was Met, Lys, Thr, Phe, Trp/Arg based on the percent difference from the concentrations of those amino acids in FM.

Proximate composition among test diets was fairly uniform (Table 2). Protein ranged from 19.9% in Diet 1 (0% BSFL; 25% FM) to 18.3% in Diet 6 (36% BSFL; 0% FM). Dietary lipid ranged from approximately 1.2% in Diet 1 to 0.9% in Diet 6. Ash and moisture content of the test diets were 2.4–2.7% and 25–23%, respectively. On the other hand, the amino acid profile of the test diets varied significantly with increasing substitution of BSFL meal for FM (Table 3). The magnitudes of the squared differences (S_d) in amino concentrations between the control diet (Diet 1) and the BSFL meal substituted diets (2–6) were similar in pattern to the limiting order among the essential amino acids found in BSFL. Moreover, progressively larger differences in the concentrations of the non-essential amino acids Glu, Gly, and Asp were noted with increasing substitution of BSFL meal for FM. When the squared deviations in amino acid concentrations in diets 2–6 with respect to the amino acid concentrations in the control diet were summed (SS_d), the amino acid profiles of diets 2–6 increased in distance from the control diet as a function of a second order polynomial with respect to BSFL meal level in the diet (Fig. 1).

In terms of growth performance, there were no significant differences in shrimp survival with respect to level of BSFL meal inclusion in the diet, with an average survival of 91% among all treatments (Table 4). Feed intake among dietary treatments did not differ, as well (Table 4). Generally, maximum responses in the current study were observed when shrimp were fed the FM control diet (Diet 1), whereas, shrimp final weight, percent weight gain, SGR, and food conversion declined with increasing substitution of FM with BSFL meal (Table 4).

Mean final shrimp weight was most closely modeled by a quadratic function of BSFL level in the diet, however, estimates of the R_{100}, R_{95} and R_{90}, i.e., the dietary BSFL meal levels allowing 100, 95 or 90% of the maximum response were in the negative concentration range. The next parsimonious model of final shrimp weight with respect to diet BSFL level was a spline function (Table 4; Fig. 2, top panel). Hence, dietary BSFL meal levels allowing 100 to 90% of maximum mean weight after 63 days of feeding ranged from 0 to 12%. Percent weight gain, SGR, and FCR were best modeled by quadratic functions of diet BSFL level (Table 4). Dietary BSFL meal levels allowing 100 to 90% of maximum weight gain (Fig. 2., bottom panel) and SGR (Fig. 3., bottom panel) ranged from 3 to 17% and 3.5–23.5%, respectively (Table 4). The best FCR's (Fig. 3., top panel), i.e., 100–90% of the minimum FCR, were found when diet BSFL level ranged from 0.1–10.2% (Table 4).

White shrimp whole-body moisture, protein, and lipid also varied as quadratic functions of diet BSFL meal level (Table 5). Substitution levels of BSFL in the diet that resulted in maximum protein and lipid content (Fig. 4) and minimum water content (Table 5) ranged from 1.1–40.2%, 1.3–21.1%, and 2.7–61%, respectively, for 100–90% of the maximum (or minimum) response. Whole-body ash of white shrimp was not related to diet BSFL level (Table 5).

4. Discussion

This is the first study to investigate the replacement of FM with BSFL meal in diets for Pacific white shrimp. There was no difference in

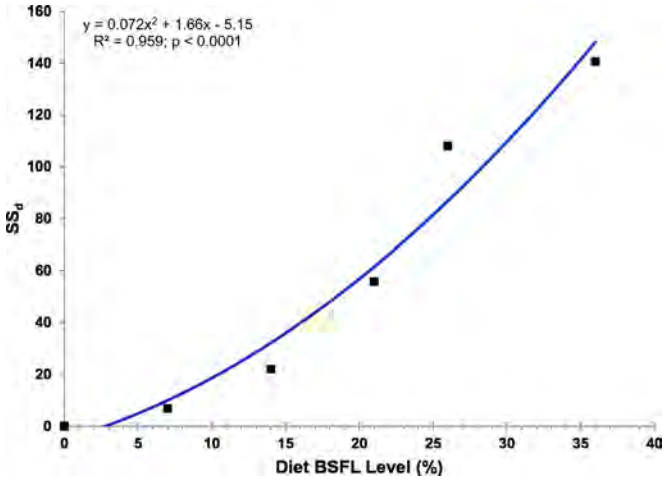

Fig. 1. Sum of the squared differences (SSd) in amino acid concentrations between the control diet containing 0% black soldier fly larvae (BSFL) meal and 25% menhaden fish meal (FM) and diets containing increasing levels of BSFL meal (7–36%) as a replacement for FM in juvenile white shrimp diets.

Table 4
Growth response of juvenile white shrimp (mean initial weight 1.24 ± 0.01; ±SE) fed six practical diets with graded levels of black soldier fly larvae (BSFL) meal as a replacement for menhaden fish meal (FM) for 63 days. Values are least squares means of $N = 3$ replicate tanks of fish per BSFL meal level (%) in the diet.

		Response[a]					
Diet	BSFL (%)	Final weight	Weight gain	SGR	Feed intake	FCR	Survival
1	0	15.95 ± 0.56	1082 ± 39	3.92 ± 0.05	30.8 ± 1.0	2.12 ± 0.15	91.1 ± 5.9
2	7	15.85 ± 0.55	1204 ± 65	4.07 ± 0.08	29.3 ± 0.9	2.01 ± 0.08	95.6 ± 2.2
3	14	13.33 ± 0.23	1017 ± 119	3.82 ± 0.16	30.9 ± 1.6	2.56 ± 0.13	91.1 ± 5.9
4	21	12.68 ± 0.67	1045 ± 137	3.85 ± 0.19	29.8 ± 1.7	2.61 ± 0.28	91.1 ± 5.9
5	26	9.30 ± 0.58	616 ± 33	3.12 ± 0.07	32.1 ± 1.9	4.02 ± 0.10	86.7 ± 3.9
6	36	8.08 ± 0.83	566 ± 33	3.06 ± 0.08	30.6 ± 1.7	4.51 ± 0.35	91.1 ± 5.9
Model[b]		spline	quadr	quadr	*NR*	quadr	*NR*
Regression analysis							
R^2		0.902	0.698	0.758	–	0.860	–
Pr > *F*		<0.0001	<0.0001	<0.0001	–	<0.0001	–
Parameter estimates[c]							
b_o		15.95	−0.56	−0.001	–	0.002	–
b_1		1.42E-16	3.20	0.007	–	−0.0004	–
b_2		−0.27	1128.52	3.980	–	2.040	–
$x_ø$		6.14	–	–	–	–	–
R_{100}		6.14	2.85	3.50	–	0.10	–
R_{95}		9.11	12.89	17.75	–	7.23	–
R_{90}		12.07	17.05	23.50	–	10.20	–

[a] Model types are quadratic (quadr), where $y = b_o x^2 + b_1 x + b_2$; or spline, where $y = b_o + b_1 x$ when $x < x_ø$ (i.e., x knot) or $y = (b_o - b_2 x_ø) + (b_1 + b_2)x$, when $x \geq x_ø$. *NR* denotes "no relationship", i.e., $P > 0.05$ and $R^2 < 0.25$ for any regression relationship explored.
[b] Model parameters as defined in footnote b above. Values R_{100}, R_{95} and R_{90} are the dietary BSFL meal levels (g BSFL/100 g diet) required to reach 100, 95 or 90% of the maximum or minimum (FCR) response, respectively.
[c] Responses include: mean (±SE) final individual shrimp weight (W_f, g), weight gain (%) = $(W_f - W_i) * 100/W_i$, where W_i = initial individual shrimp weight (g); specific growth rate (SGR; %) = $(\ln W_f - \ln W_i \times 100) / t$ where t is time in days; total feed intake (g dry); feed conversion ratio (FCR) = dry feed intake (g)/final weight gain (g), and survival (%) after 63 days.

survival percentage in the present study for any dietary treatments, which ranged from 87% to 96% among treatments. All measured water quality parameters were within optimal ranges for white shrimp growth and health. While average survival in the present study was somewhat lower than other reports (Molina-Poveda and Morales, 2004; Roy et al., 2009; Morris et al., 2011; Ye et al., 2011), several of those studies used green-water culture systems where shrimp had continuous access to supplemental nutrients. Survival in the present study, however, was similar to studies in clear-water culture systems (Izquierdo et al., 2006; Liu et al., 2012; Ye et al., 2012; Bulbul et al., 2013).

Shrimp fed Diet 1 (FM control) exhibited growth comparable to, or higher than, previous studies in shrimp (Molina-Poveda and Morales, 2004; Alvarez et al., 2007; Roy et al., 2009; Markey et al., 2010; Morris et al., 2011; Sookying and Davis, 2011; Ye et al., 2011; Sanchez et al., 2012; Yue et al., 2012; Bulbul et al., 2013; Sa et al., 2013). Moreover, from visual observations of feeding, it was apparent that palatability was high among all diets, as shrimp actively and rapidly swam to diet pellets and began to consume them. Nevertheless, the data suggest that replacement of FM by BSFL meal may be limited to 20% or less without further amendments to BSFL meal or the replacement diets. In fact, reduced growth, increased FCR, and reduced whole-body protein and lipid in shrimp ensued with >7% inclusion of BSFL. The regression analyses afford estimates of the relative feasible range of FM replacement with BSFL that extends to 90% of the best possible responses under the current conditions. For most responses of economic importance, the 90% range of BSFL substitution for FM is rather narrow at 10–17%.

The diminishing performance with increasing BSFL in the test diets is most likely due to increasing essential amino acid (EAA) deficiencies as well as increasing imbalances of EAA/nonessential amino acids (NEAA) as seen in the amino acid composition data of Table 3. At the time this study was undertaken, only the requirement for Lys for Pacific white shrimp had been estimated at 16.4–20.5 g kg^{-1} diet (Xie et al., 2012), depending on allowance for leaching loss. Lys concentrations measured in the current test diets (24.0–27.9 g kg^{-1} diet), therefore, exceeded requirements by 20–36% on the upper end of the requirement spectrum, and by 46–70% on the lower end. Subsequently, requirements for Arg, Met, and Thr in white shrimp were estimated at 19.6–23.2 (Zhou et al., 2013), 6.6–9.1 (Lin et al., 2015), and 11.8–15.1 g kg^{-1} (Zhou et al., 2013), respectively, depending on leaching loss allowance. If the lower-end requirements are considered, then only Met became limiting in Diets 4–6 containing 21–36% BSFL. If upper-end requirements are considered, then Arg was limiting in Diets 4–6, Thr was limiting in Diets 3–6, and Met was limiting in all diets, including the FM control. As illustrated by the squared differences in amino acid concentrations with respect to the control diet, the levels of three nonessential amino acids (NEAA)—Asp, Glu, Gly—also became markedly imbalanced with increasing BSFL in the test diets. It is interesting to note, however, that both shrimp responses, as well as the sum of the squared deviations in amino acid concentrations from the control diet, were significantly quadratic with respect to diet BSFL level. This suggests that immediate and large improvements in shrimp performance at higher levels of BSFL inclusion are possible through supplementation of limiting EAA and balancing the NEAA/EAA ratio of the replacement diets.

Studies evaluating BSFL meal in fish diets are extremely few, but increasing. Results of the current study in shrimp are similar to several teleost studies. In rainbow trout (RBT; *Oncohynchus mykiss*), for example, fish fed a diet with 15% BSFL meal had similar final average weight, weight gain, total feed intake, and FCR as fish fed a control diet with 36% anchovy FM, whereas, fish fed a diet containing 30% BSFL meal had significantly reduced growth parameters (St-Hilaire et al., 2007). Slawski et al. (2008) reported that Nile tilapia (*Oreochromis niloticus*) fed diets with housefly larvae meal had reduced growth and increased food conversion compared to fish fed a diet containing 52% FM without fly meal. Kroeckel et al. (2012) reported that growth and feed intake decreased in juvenile turbot (*Psetta maxima*) as BSFL meal increasingly substituted (16.5–75.6%) for FM in the diet. Whole-body protein and ash of turbot, however, were not significantly different among treatments, which is similar to the body composition results of St-Hilaire et al. (2007). Those studies indicate insect meal substitution for FM did not adversely affect protein accretion, although protein retention efficiency decreased with increasing inclusion of BSFL (Kroeckel et al.,

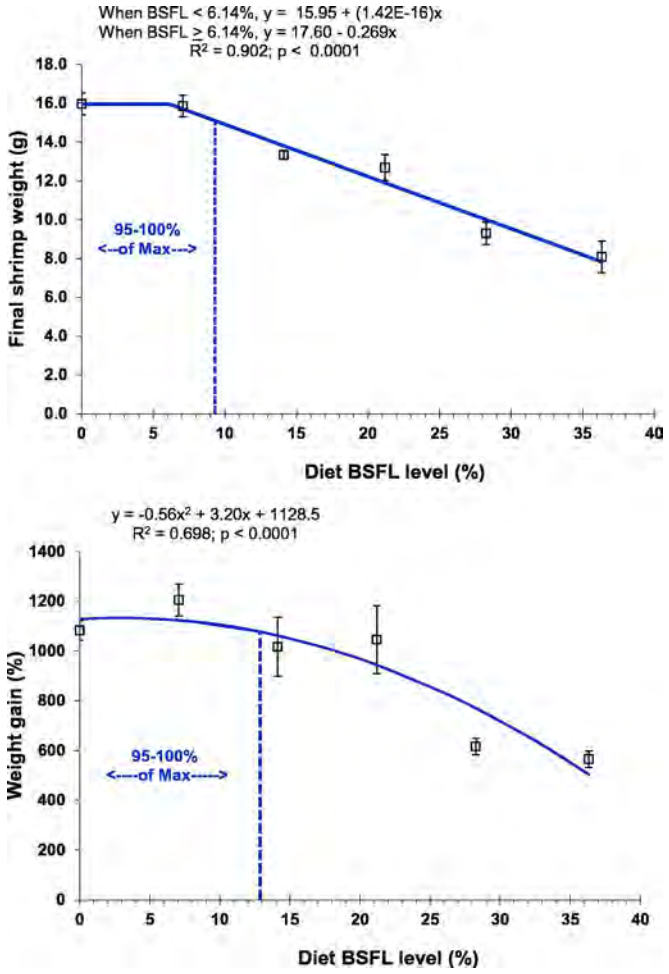

Fig. 2. Mean (±SE) final shrimp weight (top panel; spline fit) and percent weight gain (bottom panel; quadratic fit) of juvenile white shrimp (mean initial weight 1.24 ± 0.01; ±SE) fed six practical diets with graded levels of black soldier fly larvae (BSFL) meal as a replacement for menhaden fish meal (FM) for 63 days.

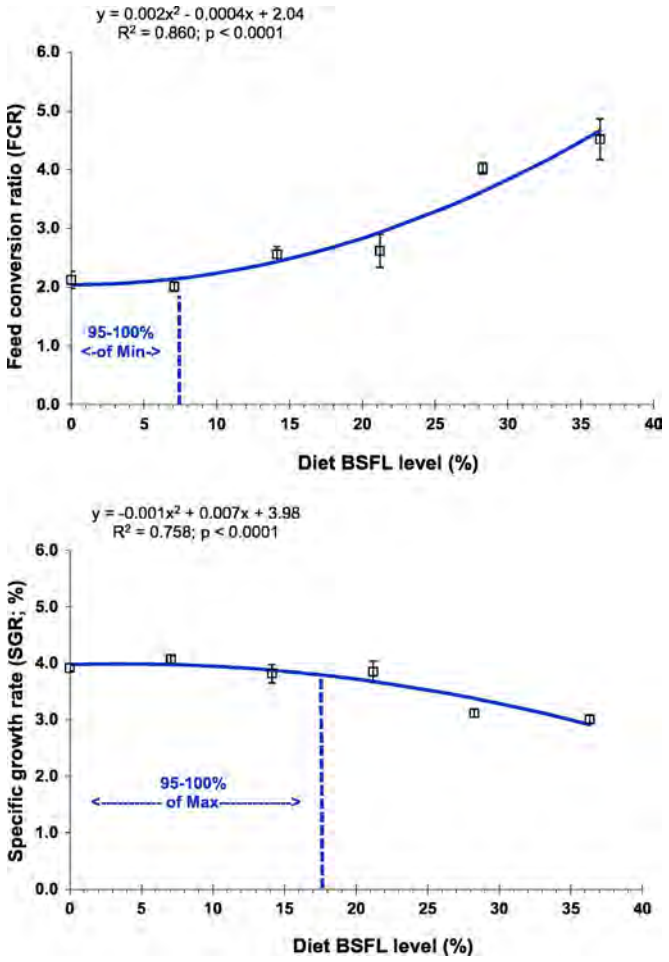

Fig. 3. Quadratic regression models of mean (±SE) specific growth rate (bottom panel) and food conversion ratio (top panel) in juvenile white shrimp (mean initial weight 1.24 ± 0.01; ±SE) fed six practical diets with graded levels of black soldier fly larvae (BSFL) meal as a replacement for menhaden fish meal (FM) for 63 days.

2012). In contrast, Fasakin et al. (2003) found that housefly (*Musa domestica*) larvae meal could totally replace FM in diets for small clariid catfish (*Clarias gariepinus*) if the meal was defatted and sun-dried.

The growth reduction seen by Kroeckel et al. (2012) in turbot fed BSFL could have been due to reduced nutrient digestibility and/or reduced diet palatability since protein digestibility for BSFL was 63.1%, which was dramatically lower than that of FM at 88–98%. Likewise, digestibility coefficients in turbot for lipid and energy in diets containing BSFL were 78% and 54.5%, respectively, compared to 98.7% and 84.9% for the diet containing FM. These lower digestibility values also could be a result of chitin in the prepupae (exoskeleton) which is indigestible to fish. Chitin can inhibit nutrient absorption from the intestinal tract resulting in reduced growth. Shiau and Yu (1999) reported, for example, that reduced lipid digestibility was observed when chitin was added to a diet for tilapia.

While fish may have varying chitinase activities, chitinase activity in marine shrimp should be sufficient to adequately digest any dietary chitin from inclusion of BSFL into the diet (Clark et al., 1993). White shrimp have mRNA Chi isoenzymes 1, 2, and 3. Moreover, LvChi1 and LvChi3 were detected in the hepatopancreas cDNA, which would suggest that these isoenzymes are actively involved in digestion of chitin in the food of this crustacean (Rocha et al., 2012). Shiau and Yu (1998) reported that addition of chitin to a diet for *Penaeus monodon* improved growth and that shrimp fed a diet containing 5% chitin had better growth than shrimp fed diets containing 2% or 10% chitin. If reduced digestibility and palatability are part of the reasons seen in the performance of insect meals in some aquaculture species, judicious diet formulation may easily overcome those issues.

Culture system environment also tends to affect results of FM substitution in shrimp diets. Feeding studies that use green water systems (ponds or tanks) where natural productivity contributes to the total nutrient intake of the shrimp, or studies where the control diet contains <15% FM, suggest that total replacement of FM is possible (Davis et al., 2002; Amaya et al., 2007a, 2007b; Roy et al., 2009; Sookying and Davis, 2011; Molina-Poveda et al., 2013). However, feeding studies that use clear-water systems, where there are no exogenous supplemental nutrient sources, indicate that complete replacement of FM is more problematic. The presence of supplemental nutrients from the culture environment or lack of a true control diet (diet with >19% FM) make comparisons among FM replacement trials difficult. Additionally, FCR of shrimp fed Diet 1 (the control) was somewhat higher than other reported values (Roy et al., 2009; Morris et al., 2011; Sookying and Davis, 2011; Ye et al., 2011; Bulbul et al., 2013), but most of those studies were carried out in green-water conditions where, as mentioned previously, natural productivity may increase growth and decrease FCR values. Feeding percentages can be reduced in green-water systems because there is continuous access to supplemental nutrients and FCR values have been reported to be much lower. The present study was conducted in a closed, recirculating system where there was no possibility of exogenous nutrients. When compared to studies conducted in clear-water systems (Molina-Poveda and Morales, 2004; Izquierdo et al., 2006; Alvarez et al., 2007; Sanchez et al., 2012; Sa et al., 2013),

Table 5
Whole-body composition of initial and final juvenile white shrimp fed six practical diets with graded levels of black soldier fly larvae (BSFL) meal as a replacement for menhaden fish meal (FM) for 63 days. Values are least squares means of $N = 3$ replicates per diet BSFL (%) meal level.

Diet	BSFL (%)	Response: Moisture	Protein	Lipid	Ash
Initial		84.22 ± 0.22	11.84 ± 0.04	0.22 ± 0.02	3.40 ± 0.09
1	0	74.94 ± 0.21	19.91 ± 0.35	1.19 ± 0.05	2.44 ± 0.06
2	7	74.87 ± 0.22	19.78 ± 0.26	1.26 ± 0.02	2.48 ± 0.06
3	14	75.26 ± 0.40	19.71 ± 0.24	1.14 ± 0.06	2.54 ± 0.09
4	21	75.58 ± 0.32	19.38 ± 0.17	1.06 ± 0.02	2.56 ± 0.14
5	26	76.38 ± 0.59	18.86 ± 0.27	0.98 ± 0.14	2.47 ± 0.01
6	36	77.35 ± 0.57	18.25 ± 0.47	0.85 ± 0.06	2.69 ± 0.09
Model[a]		quadr	quadr	quadr	*NR*
Regression analysis					
R^2		0.730	0.661	0.649	–
Pr > F					
Parameter estimates[b]					
	b_o	0.0022	−0.0013	−0.0003	–
	b_1	−0.0119	0.0028	0.0008	–
	b_2	74.915	19.892	1.222	–
	R_{100}[b]	2.7	1.1	1.3	–
	R_{95}[b]	43.9	28.7	15.1	–
	R_{90}[b]	61.0	40.2	21.1	–

[a] Model type is quadratic (quadr), where $y = b_o x^2 + b_1 x + b_2$. *NR* denotes "no relationship", i.e., $P > 0.05$ and $R^2 < 0.25$ for any regression relationship explored.
[b] Model parameters as defined in footnote a above. Values R_{100}, R_{95} and R_{90} are the dietary BSFL meal levels (g BSFL/100 g diet) required to reach 100, 95 or 90% of the maximum response, respectively.

FCRs reported in the present study are similar. Additionally, in a culture system where there are no exogenous sources of nutrients, it may be better to overfeed so as not to limit access to food and thereby artificially reduce growth rates.

Feed intake in shrimp tends to be inversely related to FM replacement by plant-protein sources (Lim and Dominy, 1990; Forster et al., 2003). In the present study, a pre-determined feeding regime was employed, and we did not attempt to measure actual intake. Hence, it is not possible to make comparisons of intake among dietary treatments. However, FCR increased in dietary treatments 2 to 6 compared to shrimp fed the control diet with FM (Diet 1). This may be the result of reduced intake/overfeeding as BSFL meal increased in the diet as suggested by the reduced final weights of shrimp fed Diets 2–6. Although the feeding rate established a priori may have been too high, the calculated feeding percentages were reasonable, nevertheless.

In conclusion, the current results give insight into current limitations of using black solder fly larvae meal in white shrimp and possible avenues of expanding insect meal replacement of fishmeal in aquaculture diets. With judicious modifications to diet formulations which include BSFL meal, increased inclusion levels may be possible in diets for Pacific white shrimp.

Acknowledgements

The authors thank N. Ann, K.C., K.N. Dee, Lyd Dia, P.O.P. Ins, B.R. Lee, T.L. Lee, E.M. Maa, Cathy Rhin, B. Rett, Shi Ron, M.S. Tee, D.R. Wynne, and Sam Wise for their technical assistance. Vaun Cummins would like to thank Sarah, Coby, and Theo for their unwavering support and inspiration; Magnus, Tar, and Copper for their companionship; and the staff, faculty and students at KSU for their technical assistance. This manuscript is in partial fulfillment of a Master's of Science degree, Division of Aquaculture, Kentucky State University for the lead author. Use of trade or manufacturer's name does not imply endorsement. Research was partially funded by a USDA grant under agreement KYX-80-09-18A to Kentucky State University.

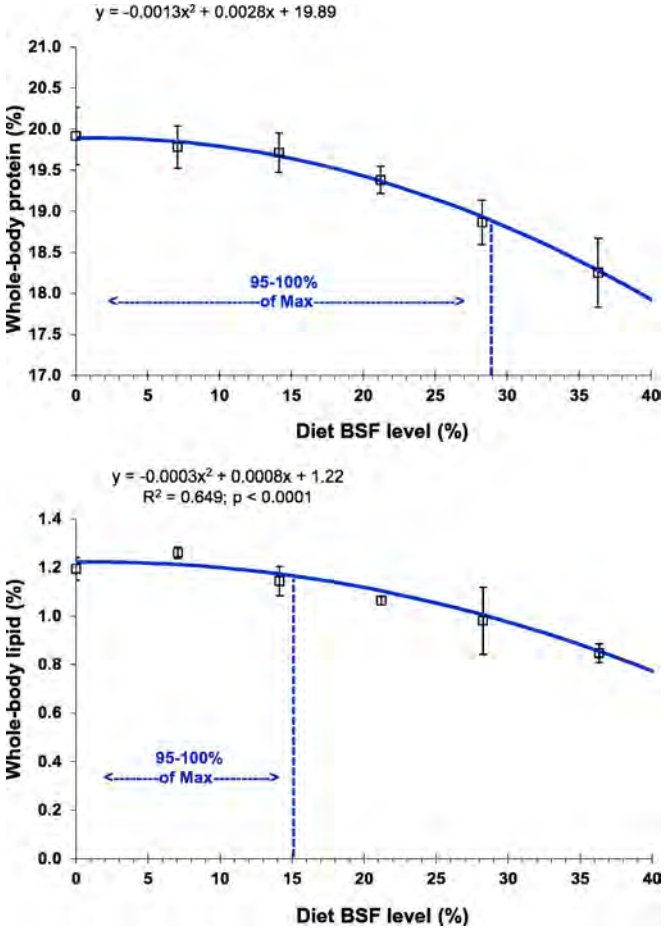

Fig. 4. Quadratic regression models of mean (±SE) whole body protein (top panel) and lipid (bottom panel) in juvenile white shrimp (mean initial weight 1.24 ± 0.01; ±SE) fed six practical diets with graded levels of black soldier fly larvae (BSFL) meal as a replacement for menhaden fish meal (FM) for 63 days.

References

Akiyama, D.M., Coelho, S.R., Lawrence, A.L., Robinson, E.H., 1989. Apparent digestibility of feedstuffs by the marine shrimp *Penaeus vannamei* Boone. Nippon Suisan Gakkaishi 55, 91–98.
Alvarez, J.S., Henandez-Llamas, A., Galindo, J., Fraga, I., Garcia, T., Villareal, H., 2007. Substitution of fishmeal with soybean meal in practical diets for juvenile white shrimp, *Litopenaeus schmitti* (Perez-Farfante & Kensley 1997). Aquac. Res. 38, 689–695.
Amaya, E.A., Davis, D.A., Rouse, D.B., 2007a. Replacement of fish meal in practical diets for the Pacific white shrimp *Litopenaeus vannamei* reared under pond conditions. Aquaculture 262, 393–401.
Amaya, E.A., Davis, D.A., Rouse, D.B., 2007b. Alternative diets for the Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture 262, 419–425.
Bauer, W., Prentice-Hernandez, C., Tesser, M.B., Wasielesky, W., Poersch, L.H.S., 2012. Substitution of fish meal with microbial floc meal and soy protein concentrate in diets for the Pacific white shrimp *Litopenaeus vannamei*. Aquaculture 342-343, 112–116.
Bondari, K., Sheppard, D.C., 1981. Soldier fly larvae as feed in commercial fish production. Aquaculture 24, 103–109.
Bondari, K., Sheppard, D.C., 1987. Soldier fly, *Hermetia illucens* L., as feed for channel catfish, *Ictalurus punctatus* (Rafinesque) and blue tilapia, *Oreochromis aureus* (Steindachner). Aquac. Fish. Manag. 18, 209–220.
Bulbul, M., Koshio, S., Ishikawa, M., Yokoyama, S., Kader, Md.A., 2013. Performance of kuruma shrimp, *Marsupenaeus japonicas,* fed diets replacing fishmeal with a combination of plant protein meals. Aquaculture 372-375, 45–51.
Clark, D.J., Lawrence, A.L., Swakon, D.H.D., 1993. Apparent chitin digestibility in penaeid shrimp. Aquaculture 109, 51–57.
Cruz-Suarez, L.E., Nieto-Lopez, M., Guajardo-Barbosa, C., Tapia-Salazar, M., Sholz, U., Ricque-Marie, D., 2007. Replacement of fishmeal with poultry by-product meal in practical diets for *Litopenaeus vannamei*, and digestibility of the tested ingredients and diets. Aquaculture 272, 466–476.
Cummins, V.C., Webster, C.D., Thompson, K.R., Velasquez, A., 2013. Replacement of fish meal with soybean meal, alone or in combination with distiller's dried grains with solubles, in practical diets for Pacific white shrimp, *Litopenaeus vannamei*, grown in a clear-water culture system. J. World Aquacult. Soc. 44, 775–785.

Davis, D.A., Arnold, C.R., McCallum, I., 2002. Nutritional value of feed peas (*Pisum sativum*) in practical diet formulations for *Litopenaeus vannamei*. Aquacult. Nutr. 8, 87–94.
FAO, 2012. Fishery and Aquaculture Statistics 2010. Food and Agriculture Organization of the United Nations. Fisheries and Aquaculture Department, Rome, Italy Fasakin, E.A., Balogun, A.M., Ajayi, O.O., 2003. Evaluation of full-fat and defatted maggot meals in the feeding of clariid catfish *Clarias gariepinus* fingerlings. Aquacult. Res. 34, 733-738.
Fasakin, E.A., Balogun, A.M., Ajayi, O.O., 2003. Evaluation of full-fat and defatted maggot meals in the feeding of clariid catfish *Clarias gariepinus* fingerlings. Aquacult. Res. 34, 733–738.
Forster, I., Dominy, W., Obaldo, L., Tacon, A.G.J., 2003. Rendered meat and bone meals as ingredients of diets for shrimp *Litopenaeus vannamei* (Boone, 1931). Aquaculture 219, 655–670.
Fox, J.M., Lawrence, A.D., Smith, F., 2004. Development of a low-fish meal formulation for commercial production of *Litopenaeus vannamei*. In: Cruz-Suarez, L.E., Ricque-Marie, D., Nieto Lopez, M.G., Villareal, D., Scholz, U., Gonzales, M. (Eds.), Avances en Nutricion Acuicola VII. Memorias del VII Simposium Internacional de Nutricion Acuicola, 16–19 Noviembre, 2004. Hermosillo, Sonora, Mexico, pp. 238–258.
Francis, G., Makkar, H.P.S., Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199, 197–227.
Gatlin, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G., Krogdahl, A., Nelson, R., Overturf, K., Rust, M., Sealy, W., Skonberg, D., Souza, E.J., Stone, D., Wilson, R., Wurtele, E., 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquac. Res. 38, 551–579.
GlobeFish, 2015. Lower Prices Helped Shrimp Consumption in USA. FAO, Rome, Italy www.fao.org/in-action/globefish/market-reports/resource-detail/en/c/417580.
Hardy, R.W., 2010. Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. Aquac. Res. 41, 770–776.
Izquierdo, M., Forster, I., Divakaran, S., Conquest, L., Decamp, O., Tacon, A., 2006. Effect of green and clear water and lipid source on survival, growth and biochemical composition of Pacific white shrimp *Litopenaeus vannamei*. Aquac. Nutr. 12, 192–202.
Ju, Z., Deng, D.-F., Dominy, W., 2012. A defatted microalgae (*Haematococcus pluvialis*) meal as a protein ingredient to partially replace fishmeal in diets of Pacific white shrimp (*Litopenaeus vannamei*, Boone, 1931). Aquaculture 354–355, 50–55.
Kroeckel, S., Harjes, A.G.E., Roth, I., Katz, H., Wuertz, S., Susenbeth, A., Schulz, C., 2012. When a turbot catches a fly: evaluation of a pre-pupae meal of the black soldier fly (*Hermetia illucens*) as fish meal substitute-growth performance and chitin degradation in juvenile turbot (*Psetta maxima*). Aquaculture 364-365, 345–352.
Kvalseth, T.O., 1985. Cautionary note about R^2. Am. Stat. 39, 279–285.
Langer, S., Bakhtiyar, Y., Lakhnotra, R., 2011. Replacement of fishmeal with locally available ingredients in diet composition of *Macrobrachium dayanum*. Afr. J. Agric. Res. 6, 1080–1084.
Lemos, D., Lawrence, A.D., Siccardi III, A.J., 2009. Prediction of apparent protein digestibility of ingredients and diets by *in vitro* pH-stat degree of protein hydrolysis with species-specific enzymes for juvenile Pacific white shrimp *Litopenaeus vannamei*. Aquaculture 295, 89–98.
Lim, C., Dominy, W., 1990. Evaluation of soybean meal as a replacement for marine animal protein in diets for shrimp *Penaeus vannamei*. Aquaculture 87, 53–63.
Lin, H.Z., Chen, Y.F., Niu, J., Zhou, C.P., Huang, Z., Du, Q., Zhang, J., 2015. Dietary methionine requirements of Pacific white shrimp *Litopeneaus vannamei* of three different sizes. Isr. J. Aquacult. Bamidgeh 67, 1–10.
Liu, X., Ye, J., Wang, K., Kong, J., Yang, W., Zhou, L., 2012. Partial replacement of fish meal with peanut meal in practical diets for Pacific white shrimp, *Litopenaeus vannamei*. Aquac. Res. 43, 745–755.
Markey, J.C., Amaya, E.A., Davis, D.A., 2010. Replacement of poultry by-product meal in production diets for the Pacific white shrimp, *Litopenaeus vannamei*. J. World Aquacult. Soc. 41, 893–902.
Molina-Poveda, C., Morales, M.E., 2004. Use of a mixture of barley-based fermented grains and wheat gluten as an alternative protein source in practical diets for *Litopenaeus vannamei* (Boone). Aquac. Res. 35, 1158–1165.
Molina-Poveda, C., Lucas, M., Jover, M., 2013. Evaluation of the potential of Andean lupin meal (*Lupinus mutabilis* Sweet) as an alternative to fish meal in juvenile *Litopenaeus vannamei* diets. Aquaculture 410-411, 148–156.
Morris, T.C., Samocha, T.M., Davis, D.A., Fox, J.M., 2011. Cholesterol supplements for *Litopenaeus vannamei* reared on plant based diets in the presence of natural productivity. Aquaculture 314, 140–144.
Naylor, R.L., Hardy, R.W., Bureau, D.P., Chiu, A., Elliott, M., Farrell, A.P., Forester, I., Gatlin, D.M., Goldburg, R.J., Hua, K., Nichols, P.D., 2009. Feeding aquaculture in an era of finite resources. Proc. Natl. Acad. Sci. 106, 15103–15110.
Newton, G.L., Sheppard, D.C., Watson, D.W., Burtle, G.J., Dove, C.R., 2005. Using the Black Soldier Fly, *Hermetia illucens*, as a Value-Added Tool for the Management of Swine Manure. Report to the Director of the Animal and Poultry Waste Management Center. North Carolina State University, Raleigh, NC, USA (35 pp).
NRC, 2011. Nutrient Requirements of Fish and Shrimp. National Research Council, The National Academies Press, Washington, DC, USA (376 pp).
Riddick, E.W., 2013. Insect protein as a partial replacement for fishmeal in the diets of juvenile fish and crustaceans. In: Morales-Ramos, J.A., Rojas, M.G., Shapiro-Ilan, D.I. (Eds.), Mass Production of Beneficial Organisms: Invertebrates and Entomopathogens. Academic Press, Waltham, MA, USA, pp. 565–582.
Rocha, J., Garcia-Carreno, F.L., Muhlia-Almazan, A., Peregrino-Uriarte, A.B., Yepiz-Plascencia, G., Cordova-Murueta, J.H., 2012. Cuticular chitin synthase and chitinase mRNA of whiteleg shrimp *Litopenaeus vannamei* during the molting cycle. Aquaculture 330-333, 111–115.
Roy, L.A., Bordinhon, A., Sookying, D., Davis, D.A., Brown, T.W., Whitis, G.N., 2009. Demonstration of alternative feeds for the Pacific white shrimp, *Litopenaeus vannamei*, reared in low salinity waters of west Alabama. Aquac. Res. 40, 496–503.
Sa, M.V.C., Sabry-Neto, H., Cordeiro-Junior, E., Nunes, A.J.P., 2013. Dietary concentration of marine oil affects replacement of fish meal by soy protein concentrate in practical diets for the white shrimp, *Litopenaeus vannamei*. Aquac. Nutr. 19, 199–210.
Sanchez, D.R., Fox, J.M., Gatlin, D., Lawrence, A.L., 2012. Dietary effect of squid and fish meals on growth and survival of Pacific white shrimp *Litopenaeus vannamei* in the presence or absence of phytoplankton in an indoor tank system. Aquac. Res. 43, 1880–1890.
Shiau, S.Y., Yu, Y.P., 1998. Chitin but not chitosan supplementation enhance the growth of grass shrimp, *Penaeus monodon*. J. Nutr. 128, 908–912.
Shiau, S.Y., Yu, Y.P., 1999. Dietary supplementation of chitin and chitosan depresses growth in tilapia, *Oreochromis niloticus* × *O. aureus*. Aquaculture 179, 439–446.
Slawski, H., Schulz, C., Ogunji, J.O., 2008. Evaluation of housefly maggot meal as an alternative protein source in the diet of *Oreochromis niloticus*. World Aquacult. 39 (2), 16–18 72.
Sookying, D., Davis, D.A., 2011. Pond production of Pacific white shrimp *Litopenaeus vannamei* fed high levels of soybean meal in various combinations. Aquaculture 319, 232–239.
Sookying, D., Davis, D.A., Soller Dias da Silva, F., 2013. A review of the development and application of soybean-based diets for Pacific white shrimp *Litopenaeus vannamei*. Aquac. Nutr. 19, 441–448.
St-Hilaire, S., Sheppard, C., Tomberlin, J.K., Irving, S., Newton, L., McGuire, M.A., Mosley, E.E., Hardy, R.W., Sealey, W., 2007. Fly prepupae as a feedstuff for rainbow trout, *Oncorhynchus mykiss*. J. World Aquacult. Soc. 38, 59–67.
Suarez, J.A., Gaxiola, G., Mendoza, R., Cadavid, S., Garcia, G., Alanis, G., Suarez, A., Faillace, J., Cuzon, G., 2009. Substitution of fish meal with plant protein sources and energy budget for white shrimp, *Litopenaeus vannamei* (Boone, 1931). Aquaculture 289, 118–123.
Tacon, A.G.J., Metian, M., 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects. Aquaculture 285, 146–158.
Trosvik, K.A., Rawles, S.D., Thompson, K.R., Metts, L.A., Gannam, A., Twibell, R., Webster, C.D., 2012. Growth and body composition of Nile tilapia, *Oreochromis niloticus*, fry fed organic diets containing yeast extract and soybean meal as replacement for fish meal, with and without supplemental lysine and methionine. J. World Aquacult. Soc. 43, 635–647.
Webster, C.D., Goodgame-Tiu, L.S., Tidwell, J.H., Rouse, D.B., 1994. Evaluation of practical feed formulations with different protein levels for juvenile redclaw crayfish, *Cherax quadricarinatus*. Trans. Kent. Acad. Sci. 55, 108–112.
Webster, C.D., Tiu, L.G., Tidwell, J.H., Van Wyk, P., Howerton, R.D., 1995. Effects of dietary protein and lipid levels on growth and body composition of sunshine bass (*Morone chrysops* × *M. saxatilis*) reared in cages. Aquaculture 131, 291–301.
Xie, F.J., Zeng, W.P., Zhou, Q.C., Wang, H.L., Wang, T., 2012. Dietary lysine requirement of juvenile Pacific white shrimp *Litopenaeus vannamei*. Aquaculture 358–359, 116–121.
Ye, J.D., Wang, K., Li, F.D., Sun, Y.Z., Liu, X.H., 2011. Incorporation of a mixture of meat and bone meal, poultry by-product meal, blood meal and corn gluten meal as a replacement for fish meal in practical diets of Pacific white shrimp *Litopenaeus vannamei* at two dietary protein levels. Aquac. Nutr. 17, e337–e347.
Ye, J.D., Liu, X.H., Kong, J.H., Wang, K., Sun, Y.Z., Zhang, C.X., Zhai, S.W., Song, K., 2012. The evaluation of practical diets on a basis of digestible crude protein, lysine and methionine for *Litopenaeus vannamei*. Aquac. Nutr. 18, 651–661.
Yue, Y., Liu, Y., Tian, L., Gan, L., Yang, H., Liang, G., 2012. Effects of replacing fish meal with soybean meal and peanut meal on growth, feed utilization and haemolymph indexes for juvenile white shrimp *Litopenaeus vannamei, Boone*. Aquacult. Res. 43, 1687–1696.
Zhou, Q.-C., Wang, Y.-L., Wang, H.-L., Tan, B.-P., 2013. Dietary threonine requirements of juvenile Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture 392-395, 142–147.